Chapter

From Genes to Biomarkers: Understanding the Biology of Malaria Gametocytes and Their Detection

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Abstract

Each year, approximately 230 million malaria cases and 400,00 malaria deaths are reported worldwide. Malaria is a life-threatening disease caused by *Plasmodium* parasites that are transmitted from one individual to another through the bites of infected female *Anopheles* mosquitoes. Malaria parasites replicate asexually in the human host, and, in each replication cycle, a portion of the asexual stages develops into sexual gametocytes that permit transmission. The proportion of infections that carries gametocytes and the infectivity of gametocytes are indicators of human-to-mosquito transmission potential. In *P. falciparum*, gametocytes appear 10–14 days after infection, whereas in *P. vivax* gametocytes appear simultaneously with asexual schizonts. Such difference in development not only increases the length of time that an individual is infectious, but also increases the likelihood of transmission before treatment. The conversion from asexual parasites to gametocytes is also highly variable between infections. Differences in age, host immune response, parasite genetic composition, density of red blood cells, presence of co-infecting parasite strains, and antimalarial drug use could affect gametocytes production. In *P. vivax*, the unique ability to produce hypnozoites, a dormant liver stage of the parasite, may allow gametocytes to be produced periodically from relapse and contribute to transmission. In this chapter, we will provide an overview of the biology of *Plasmodium* gametocytes, existing tools for gametocyte detection, and features of gametocyte genes. The biological insights and genetic findings are essential to developing better detection biomarkers and effective strategies to reduce transmission in malaria-endemic countries.

Keywords: *Plasmodium*, gametocyte, epidemiology, biomarkers, transmission

1. Introduction

1.1 Malaria epidemiology

Malaria is a mosquito-borne disease. In humans, malaria is primarily caused by six different *Plasmodium* parasite species including *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*, and *P. simium* [1]. To date, there is an estimated 229 million cases of malaria worldwide each year resulting in approximately 409,000 deaths [2]. This has resulted in an estimated direct cost (treatments, premature death, and loss of household income) of $12 billion USD per year, with lost economic growth being many times greater [3]. The deadliest of these is *P. falciparum*, primarily located in the African continent [4], due to the high parasitemia levels and rapid growth [4]. *P. falciparum* utilizes several host receptors, including Band 3 and Glycophorin A, B, and C for red blood cell invasion [5], which contributes to high parasite loads in infected patients and spread across the ethnically diverse continent. Contrary to *P. falciparum*, *P vivax* primarily uses the Duffy antigen receptor found on the surface of reticulocytes for invasion, leading to lower parasitemia levels. *P. vivax* is globally widespread in Asia, South America, and northern and eastern parts of Africa [6]. This parasite can cause relapse from weeks to months after the initial infection [6].

Most *P. ovale* cases were reported in the tropics of western Africa [7] and is considerably less prevalent and possibly less fatal in comparison to *P. falciparum* and *P. vivax* [8]. *P. ovale* has two different subspecies: *P. ovale curtisi* and *P. ovale wallikeri*, both of which may produce dormant stage hypnozoites similarly to *P. vivax* [9, 10]. *P. ovale* has been shown to be transmitted across international borders from Africa to China [11] as well as to Korea [11, 12], in countries that were considered malaria free [12]. *P. malariae* is mostly reported in sub-Saharan Africa and southeast Asia. Similar to *P. ovale*, *P. malariae* is less common and less fatal. In sub-Saharan Africa, infections of *P. malariae* are often found to also contain *P. falciparum* [13]. Though less common, *P. knowlesi*, a primate parasite infecting long-tailed and pig-tailed macaques [14], has recently been reported to infect humans in Southeast Asia [15, 16] and is mainly transmitted by *Anopheles leucosphyrus* and *A. hackeri* [16–18]. *P. knowlesi* utilizes two gene families: DBL and RBP, to bind the Duffy antigen/chemokine receptor (DARC) to invade human erythrocytes [19]. Last but not least, *P. simium* is a common primate parasite that has recently been detected through molecular assays in humans from Atlantic Forest region of southeastern Brazil [20]. *P. simium* remains largely uncharacterized due to its novelty and rarity in humans, but is thought to have similar invasion mechanisms to *P. vivax* due to genetic and morphological similarities [20]. The presence of these less-common malarial species emphasizes the need for better detection tools and control measures especially in countries approaching the elimination phase.

1.2 Development and transmission of *Plasmodium*

The lifecycle of *Plasmodium* is divided into two stages: the human asexual reproduction stage (also known as the erythrocytic stage) and the mosquito sexual reproduction stage (known as the sporogonic cycle) [20], both of which are necessary for population growth and genetic diversification [19]. The parasite first enters the human body in the form of sporozoites through the infected salivary gland of an *Anopheles* mosquito during a blood meal. Within minutes, sporozoites infect the liver and begin asexual replication to form schizonts (a process called exoerythrocytic schizogony) within a few days to a few weeks [20]. Once matured, schizonts bust to release hundreds of merozoites into the blood stream [20]. *P. falciparum*, *P. ovale*, *P. malariae*, and *P. simium* infect mature erythrocytes [2, 19, 21] while *P. vivax* and *P. knowlesi* infect younger reticulocytes [2, 19, 22–24] to feast on

hemoglobin and further reproduce asexually [2, 19]. At this stage, the intracellular *Plasmodium* is considered as a ring stage parasite because the young parasite takes up hemoglobin in a single, large vacuole that looks like a ring. Once the hemoglobin is engulfed, the parasite then begins feeding and hemoglobin is acquired by endocytosis of erythrocyte cytoplasm within cytostomes, known as the trophozoite stage. After the hemoglobin is consumed, the trophozoite may either undergo schizogony to asexually reproduce and start the erythrocytic cycle with new merozoites or mature into a macrogametocyte (egg) or microgametocyte (sperm). Because human body temperature is considerably warm for *Plasmodium* sexual reproduction [25], both forms of gametocytes are taken up by an *Anopheles* mosquito where sexual reproduction occurs within the gut of the mosquito producing zygotes. Once matured, the zygote will become mobile and elongated, developing into ookinete to infect the midgut wall of the mosquito [26] and develop further into oocyst. Oocysts asexually divide and eventually rupture into sporozoites that travel to the salivary gland of the mosquito to infect a new human host [2, 19].

All six human malaria parasites require gametocytes to infect female *Anopheles* mosquito to reproduce sexually and continue development into sporozoites before infecting a new human host [27]. This has led to complications in the control of malaria transmission and gametocyte detection [28]. Furthermore, *P. vivax* and *P. ovale's* unique ability to form dormant stage hypnozoites complicates parasite clearance and can reintroduce old parasite strains into transmission reservoirs [2, 19]. Gametocytes are critical for malaria transmission and possible immune evasion in both the human and mosquito hosts [29, 30], emphasizing the importance of accurate detection. This chapter aims to provide a systemic review that highlights the complexity of *Plasmodium* gametocytes at the biological and genetic levels and current methods used to track and detect *Plasmodium* gametocytes. We further examine the capability of biomarkers used across *Plasmodium* species and provide new candidate biomarkers to further enhance detection protocols. Lastly, we examine the immunogenicity of gametocytes in both humans and mosquitoes.

2. Gametocytogenesis

2.1 Gametocyte commitment and development

Gametocytogenesis is the commitment of a *Plasmodium* parasite to produce male and female gametocytes through mitotic division during the trophozoite stage that involves multiple epigenetic and transcriptional regulations [31]. Changes in temperature, pH, and host age help stimulate gametogenesis, the emergence of the gametes in the mosquito mid-gut. A drop in temperature from 38°C to 20-26°C, the exposure to gametocyte activating factors and/or mosquito exflagellation factors, as well as a rise in pH can trigger gametogenesis [32–34]. Some merozoites, for reasons not fully understood, differentiate into the sexual forms of the parasites, the gametocytes. Upon ingestion by the mosquito, the decline in pH, a drop in temperature, and other mosquito derived factors such as xanthurenic acid can together activate gametocytes to transform within 5–10 minutes to male (microgamete) and female gametes (macrogamete) within the mosquito midgut. When gametocytes are taken up during a mosquito's blood meal, a number of factors including temperature, oxygen and carbon dioxide concentration, pH and exflagellation factor contribute to the maturation of gametocytes [35]. Some species, such as *P. ovale* in humans and *P. yoelii* in mice, can develop into morphologically distinct male and female gametocytes directly from hepatic merozoites for further transmission [7]. Gametocyte commitment is largely based on stress factors including high parasitemia, anemia,

drug treatments, and host immune responses [36–39]. Although no clinical symptoms are experienced during gametocytogenesis, this developmental stage is critical for sexual replication in the mosquitoes and subsequent infection of a new human host in the form of sporozoites. There is considerable variation in the development time among the different human *Plasmodium* species, ranging from 7 to 10 days after the initial establishment of asexual parasites for *P. falciparum* [40] and 7–15 days for *P. vivax* [41]. It is yet unclear about the time for gametocyte development in *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. simium.* During this prolonged maturation period, gametocytes undergo five morphologically distinct stages (I-V) [42]. The immature gametocytes developed during stages I-IV sequester mostly in host tissues, particularly in bone marrow and spleen [43]. At stage V during maturation, gametocytes become more deformable and return in the blood circulation for uptake by a new mosquito host [44]. For proper sporogonic development to occur, both male and female gametes must be taken up during a blood meal as merozoites are incapable at forming both male and female gametocytes from the same schizont [45]. Most regulation is performed through RNA binding proteins, such as acetylation lowers binding efficiency 4 (ALBA4), that increases exflagellation events in an unknown manner [46, 47].

To date, most of our knowledge on gametocyte commitment is derived from *P. falciparum* due to a lack of viable culturing methods for *P. vivax* [48] and low prevalence of other *Plasmodium* species. Molecular processes including epigenetic regulation and histone posttranslational modifications play vital roles in gametocyte commitment [49–51]. A well-studied example of histone regulation in *P. falciparum* is H3K9me3, which is normally restricted to multigene families in subtelomeric regions [52], and recruits heterochromatin protein 1 (HP1) [53, 54]. Both proteins are strongly associated with the *AP2-G* locus, the primary regulator for gametocytogenesis [55, 56]. Previous studies have shown that a majority of asexual parasites have *AP2-G* silenced [47] and this gene silencing may be induced by histone deacetylase (Hda2) [57]. Depletion of HP1 through *GDV1*, another gene that regulates gametocyte production, was shown to increase schizont development into gametocytes by 50% [47, 58]. Further, the presence of Stabilization ligand Shield 1 (Shld1) may also increase transcription of *AP2-G* by stabilizing the protein complex for transcription and enhance gametocyte production. Although the molecular mechanisms of gametocytogenesis are well established in *P. falciparum*, there are currently no sensitive and reliable methods available in clinical settings for front-line detection of *in vivo* gametocytes due to strain polymorphisms, limiting gametocyte densities, and variations in the timing of gametocyte production and development.

2.2 Polymorphisms of gametocyte genes

Recently, advances in next generation sequencing provide new insights in our understanding of genetic variation and gene expression across different stages of *Plasmodium* species [59]. The mosquito immune system is a significant barrier for some *Plasmodium* isolates to infect the mosquito hosts. For example, in *P. falciparum*, highly expressed Pfs47 protein allows the parasites to become "invisible" and escape the immune system of the mosquito. This protein is expressed on the surface of female gametocytes and ookinetes. The African isolates of *P. falciparum* that are known to express Pfs47 have been shown to escape the *Anopheles gambiae* immune system by suppressing the Jun-N-terminal kinase (JNK) signaling and avoiding the induction of epithelial nitration [59]. The Pfs47 protein gene is highly polymorphic implying that the mosquito immune system may be the driving force for diversity, and that parasites with compatible Pfs47 haplotypes and/or escape immune evasion are preferably selected [60].

The innate immune response in the mosquito vector is mediated in most part by the hemocytes, which eliminate pathogens such as bacteria, fungi, and protozoa. *Anopheles* mosquitoes are known to have a complement C3-like protein called thioester-containing proteins (TEP). TEP of *An. gambiae* (AgTEP1) has been shown to initiate immune defense against *P. berghei* [61]. TEP1 facilitates the interaction between the parasite and hemocytes with late encapsulation that kill the parasite. Knockdown of the TEP1 gene renders genetically selected refractory *Anopheles* strain susceptible to infection and increases the infectivity rates [61]. However, *P. falciparum* has been shown to bypass the TEP-based defense mechanism using its 6-cystein protein P47-like [61]. In *P. berghei*, the P47-like protein is important for female gamete fertility, whereas in *P. falciparum*, it promotes the gametocyte-to-ookinete development and protects the ookinete from complement-dependent lysis [61]. Furthermore, infection of *An. gambiae* mosquitoes by ookinetes of *P. berghei* has been shown to module the mosquito's immune system by up-regulating expression of the peptide defensin and a putative gram-negative bacteria-binding protein and a TNF-α factor-like transcription factor (LL3) [61].

2.3 Expression of gametocyte genes

During the different stages of gametocyte development, various genes express differentially. For instance, *Pfs*16 expresses the earliest and highest in stage II gametocytes, the alpha-tubulin II gene shows maximum expression levels in both stage II and III gametocytes, *Pfs*230 expresses in stage III gametocytes, PfsMR5 in stage IV gametocytes, and *Pfs*28 in stage V gametocytes [62]. Such gene expression pattern is directly related to the activities involved in each specific stage. *Pfs*16 is known as a marker for detecting sexually committed ring stages parasites and it likely plays a key role in gametocyte maturation given its continual expression during the entire gametocyte maturation process [63]. Recent immunofluorescences assay identified a small population of schizonts that expressed the *Pfs*16 gene. *Pfs*16 mRNA increases in the asexual cycle before schizonts develop into stage I gametocytes, though translation is delayed until the onset of stage I gametocytogenesis [62]. This finding suggests that both *Pfs*16 transcription and translation may begin prior to invasion of a committed merozoite and development into a stage I gametocyte.

The female gametocyte specific gene *Pfs*25, which expresses solely in the mosquito, is a glycosylphosphatidylinositol-linked protein expressed on the surface of ookinetes [64]. This gene is used for the detection of stage V female mature gametocytes as well as in the quantification of gametocytes from field studies [63]. Because *Pfs*25 is solely expressed inside the mosquito hosts with limited immune selective pressure, sequence variation between isolates is relatively low [65]. Antibodies against *Pfs*25 have been shown to reduce oocyst production in *in vitro* membrane feeding experiments. Both high sequence conservation and antibody response make *Pfs*25 a leading target for transmission-blocking vaccine design. Apart from *Pfs*25, another family of gametocyte protein with cysteine-rich domains includes *Pfs*230 and *Pfs*48/45 that have also been targets of transmission-blocking vaccine development [66, 67].

*Pfs*230 expresses in both male and female gametocytes with a prodomain that is processed during gametocytogenesis and mediates red blood cell binding, specifically during oocyst development [68]. The Pfs230 protein appears on the surface of gametes as a complex with Pfs48/45 (a glycosylphosphatidylinositol (GPI) anchored protein [69]) and appears to be critical for gamete fusion. The double domains in Pfs230 reveal a structure resembling the surface antigen 1 (SAG1) protein with a double beta-sandwich structure found in *Toxoplasma gondii*, another apicomplexan parasite. The complete gene encoding the Pfs230 protein from different isolates of *P. falciparum* showed that 27 nonsynonymous polymorphic sites [70]. Among

them, the amino acids at eight polymorphic sites map to positions that point their side chains toward the surface of the protein [70]. Five of those eight sites map to a confined region on the same side of the beta-sandwich of the protein structure. Even though the distribution of the amino acids are over much of the length of the domain IV sequence, the polymorphic sites appear to outline, although loosely, a contiguous surface region of the model [70]. This structural feature may provide relevant sites with respect to the interaction of male and female gametes [70].

In both male and female gametocytes, *Pfs*230 is expressed without a known membrane anchor and appears as a complex with the Pfs48/45 proteins on the surface of gametes, though this colocalization is not required. Pfs230 has been observed on the surface of live macrogametes in the absence of Pfs48/45 [71]. Pfs48/45 is a cysteine rich surface protein that is vital in male gamete fertility. Sequence analyses of *Pfs*48/45 revealed that polymorphisms are rare for residues involved at the binding interface [72]. Furthermore, unlike other pre-erythrocytic blood stage antigens Pfs48/45 is less polymorphic. The number of synonymous substitutions per synonymous site exceeds the number of non-synonymous substitutions per non-synonymous site [73].

Recent clustering analyses indicated that there is a clear distinction in the expression levels between male and female gametocyte genes. Male and female gametocytes have been shown to be differentiated by their gene expression levels as early as stage III. The female gametes are generally separated by stage, indicating differentiating gene expression levels with respect to mid and late-stage female gametocytes [74]. For male gametocytes, PF3D7_1325200, a putative lactate dehydrogenase gene, is highly expressed [74]. Another male gametocyte gene PF3D7_1311100 is a putative meiosis-specific nuclear structure protein 1. This gene is essential for normal assembly of the sperm flagella in mice, suggesting that it may have a role in the male gamete development and exflagellation. Other genes including Pf3D7_1114000, Pf3D7_1122900, Pfg14–748, HAP2, and MAPK2 are found to be associated with male gametocytes, though both Pf3D7_1114000 and Pf3D7_1122900 are also expressed in a few female gametocytes [74].

3. Genetic markers for detection and prevention

3.1 Conventional genes used for gametocyte detection

Previous study has indicated that approximately 10% of *P. falciparum* and 60% of *P. vivax* infections had concurrent detectable low-density gametocytemia [75]. Gametocyte densities are typically lower than asexual parasite densities (**Figure 1A**) and directly associated with total asexual parasite densities (**Figure 1B**). Given the relatively low gametocyte densities, it is conceivable that many of these infections are submicroscopic and remain undetected in communities where malaria occurs, contributing to continuous transmission.

Molecular tests for diminutive amounts of gametocytes use reverse-transcription polymerase chain reaction (RT-PCR) to amplify RNA transcripts of gametocyte-specifically expressed genes. Compared to DNA-based assay, qRT-PCR of targeted RNA transcripts revealed higher sensitivity in detecting gametocytes of considerably low densities. For example, there are more than 10^6 copies of 18S rRNA transcript per cell but only 5 copies of the 18 s rRNA gene per genome [76]. The production of high transcript copies in parasite cells allow for greater detection limits. For *P. falciparum*, several targeted genes such as *Pfs*25, *Pfg*136, *Pfg*84, and *Pfg*17 are specific to female gametocytes. The lack of genes specific to male gametocytes could underestimate the total gametocytes in an infection and thus

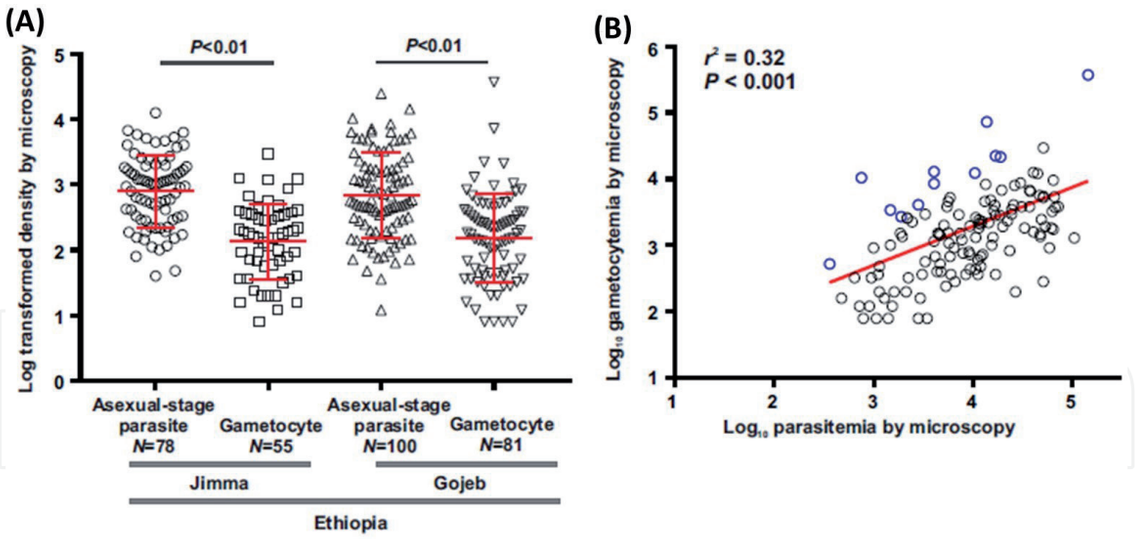

Figure 1.
(A) Comparison of asexual parasitemia and gametocytemia among P. vivax *infections from Ethiopia. (B) Significant correlation was observed between asexual parasitemia and gametocytemia.*

its transmission potential. Moreover, genes often show wide variations in detection limit based on the level of RNA transcript production, ranging from 100 gametocytes per microliter of blood by *Pfg*84, 70.7 gametocytes/μL by *Pfg*136, 25.3 gametocytes/μL by *Pfs*25 to as low as 10 gametocytes/μL by *Pfg*17 [77]. *Pfg*17 is considered as the most sensitive biomarker so far because it can detect the lowest number of gametocytes. For example, in malaria-endemic areas of Ghana, *Pfg*17 offers higher sensitivity than *Pfs*25 in detecting *P. falciparum* gametocytes in clinical samples collected from children and adults [61]. Of the 80 children, 47 were tested negative and 33 were positive for asexual blood stage *P. falciparum.* Nine out of the 47 (19%) negative children were detected positive for *P. falciparum* gametocytes by Pfg17. Among 30 adults aged from 32 to 60 years, 21 were (70%) tested positive for *P. falciparum* gametocytes by *Pfg*17, but only 7 (23.3%) were positive by *Pfs*25 [61].

For *P. vivax*, *Pvs* 25 and Pvs16 that are specific to female gametocytes are two conventional gene markers for gametocyte detection [78]. One gametocyte roughly corresponds to four *Pvs*25 transcripts per cell [79], and *Pvs*25 can detect from a mean of 0.34 gametocytes per μL blood from *P. vivax* patients in Papua New Guinea [78] to a mean of 2 gametocytes per μL blood in patients from Ethiopia [75]. Such low gametocyte densities make them extremely difficult to be detected by microscopy. The number of *Pvs*25 gene transcript copes detected by qRT-PCR directly correlates with the number of mature gametocytes as well as the overall parasite densities [41, 80] and was shown a nearly normal distribution with a mean of 1.2×10^7 copies/μL (ranging from 1.1 to 4.8×10^8 copies/μL) blood among 42 symptomatic *P. vivax* patients from northwestern Brazil [81]. Prior studies showed that age is tightly associated with gametocytemia. A lower proportion of infections with gametocytes was found with increasing age [79, 82]. Gametocytes are generally detected in ~20% of the infections among adults [41], but at much higher proportions in children under the age of 12 [82, 83]. Yet, gametocytemia in adults is up to 20-fold higher than in children [84, 85]. In areas with low levels of transmission, a large proportion of infections that are undetected by microscopy could be reservoir for parasites with high infectious gametocytes [86]. In Ethiopia, symptomatic *P. vivax* infections are nearly four times more infectious than asymptomatic ones [87]. Other factors such as host immune response, parasite strains, red blood cell density, antimalarial drug use, and relapse can also affect gametocyte production [79, 88, 89]. For *P. malariae* and *P. ovale*, there is yet no gametocyte assays due to little success in finding *Pfs*25 or *Pvs*25 orthologues in these species.

3.2 Novel gene candidates to improve detection sensitivity

Recently, the male-specific gene *pfs*13 that offers the lowest female to male expression ratio was identified as a new male gametocyte biomarker used in qRT-PCR assays from field isolates [90]. Other male-specific markers including PF3d7_1311100, PF3D7_1325200, and Pfg14–748 were also shown with abundant stage V male gametocytes in infected samples that made them as sensitive biomarker candidates [74], although their specific functions are unclear. Furthermore, *CCp*1, *CCp*3, and *P25* were identified as better biomarkers in differentiating female from male gametocytes [74, 91]. These genes are highly expressed in stages III to V female gametocytes. Though another gene *NEK*4 is female-specific, it is not sufficiently expressed in the transcriptome until stages IV and V, suggesting that it could be a good late-stage female biomarker candidate. PF3d7_1107800, a putative AP2 transcription factor, also strongly correlates as a female specific gametocyte gene similar to *NEK*4. PF3d7_1107800 is highly expressed only during stages IV and V, and thus, could potentially a biomarker for late-stage female gametocyte detection [74]. Besides, female gametocyte marker gene *CCp*4 was also identified as a new target for gametocyte detection [92]. The design of intron-spanning primers of these novel genes allows for the amplification of mRNA only without a DNA digestion step [92].

A recent study of 26 *P. vivax* samples from Cambodian patients indicated that the expression profile of 21 predicted gametocyte genes were clustered in two distinct groups [93]. One group includes *Pvs*25, *ULG*8, gametocyte developmental protein 1, guanylate kinase, *HMGB*1, and five CPW-WPC proteins that associate with intracellular trafficking and histone remodeling in the female gametocytes. The other group includes *Pvs*47, *Pvs*48/45. *Hap*2, the gamete egress and sporozoite traversal protein, s16, and three CPW-WPC proteins that associate with microtubular development in the male gametocytes. It remains to be determined if these male and female gametocyte genes show higher expression than the conventional marker *Pvs*25, offer high detectability of the total potentially transmitting gametocyte densities (both male and female gametocytes), and allow for robust gametocyte sex-ratio estimates in field studies given their stability under suboptimal storage conditions [92].

3.3 Treatment and prevention

In *P. falciparum* infections, gametocytes express surface antibodies against the surface antigens for circumsporozoite protein (PfCSP) 2A10, apical membrane antigen 1 (PfAMA1), and thrombospondin-related adhesive protein (TRAP). In *P. vivax* infections, Pvs48/45 and PfCLAG9 have been shown to elicit naturally acquired immune responses [65, 66]. Gene 1613 has been identified to be critical for the development and maturation of gametocytes, which could potentially impact the elicitation of humoral immune response and the ability of gametocytes to transmit [94]. These could be critical antigen proteins for vaccine design. As gametocytes mature from one stage to another, the production of antibodies in human hosts will cause gametocytes to develop and mature at a much slower rate than normal in the bone marrow [94–96]. Combining multiple antigens involved in different stages of gametocyte development and sexual progression can help interfere and diminish transmission [69]. Predicting the structure of the parasite antigen binding domains e.g., in AMA1 and Pfs48/45, and delineating epitopes targeted by the host antibodies could uncover key elements in blocking transmission and provide a benchmark for evaluating vaccine efficacy [70]. Vaccines that block the ability of gametocytes to transmit can reduce infections and lower the transmission potential from mosquitoes to humans in malaria-endemic areas [67]. Future studies should focus on expanding our understanding of gene interactions and their functions as well as disease control by way of transmission blocking vaccine

development [71]. Furthermore, uncharacterized chemotypes that possess activity against sexual parasites have also been recently identified to inhibit transmission to mosquito and human contact [68]. Interference by JmjC inhibitor known as ML324 causes expression to subsequently cease methylation activity followed by gametocyte lysis for *P. falciparum.* Identifying key surface proteins present in multi-parasite life rostrums could potentially be means of a preventative antimalarial against gametocytes [68].

4. Conclusions

To date, gametocytogenesis and gametocyte transmission tracking remain largely uncharacterized due to low prevalence and technological hurdles. As several countries approach the elimination phase for malaria, the need for sensitive and reliable biomarkers for gametocyte detection is more urgent than ever. Microscopy has low detection limit to overcome low parasitemia loads and existing qPCR biomarkers fail to accurately detect both sexes of the parasites. Information on genetic polymorphisms and expression levels of gametocyte biomarkers enable researchers to develop a more sensitive and accurate diagnostic test for *Plasmodium* gametocytes. Knowledge of gametocyte reservoirs and their interactions with host immune system will help develop effective treatment and preventive strategies to minimize the risk of malaria transmission. We recommend future studies focus on gene interactions and protein functions involved in gametocyte development, as well as polymorphisms in novel gametocyte genes that could be targets for developing better diagnostics or transmission blocking vaccines.

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